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Review Article **Open Access**

Genital mycoplasmas and gynaecologic cancer: A systematic review

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Abstract:

Studies on genital mycoplasmas (GM) role in gynaecologic cancers (GC) such as cervical, endometrial, ovarian, vaginal, vulva and fallopian tube, is limited. This review was conducted to evaluate an association between GM and GC. The systematic study was conducted in accordance with PRISMA guidelines across online databases including Embase, Google Scholar, PubMed, Scopus, and Web of Science from inception to August, 2022. We included cross-sectional and case-control studies examining possible connection of GM infection and development of GC, and all evidence-based studies with likely association between GM infection and incidence of GC were studied. Selection criteria aided identification, screening, and risk of bias assessment. Thirteen studies with at least moderate risk of bias, were included. The most commonly associated GMs was *Mycoplasma genitalium* followed by *Ureaplasma urealyticum* and *Mycoplasma hominis*. Studies reported disease advancement with GMs most especially in cases of co-infection. The most associated GCs were cervical, ovarian and endometrial. Infection with *U. urealyticum, M. hominis*, and *M. genitalium* was associated with cervical cancer risk (OR 1.31-1.41), and *M. hominis* and *M. genitalium* had associated risk with ovarian (RR 0.93-1.92) and endometrial cancer (OR 1.36- 2.07). No association was found with vaginal, vulva and fallopian tube cancers.

Keywords: Genital mycoplasma; gynaecologic cancer; cervical cancer; association; infection

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Mycoplasmes génitaux et cancer gynécologique: une revue systématique

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Résumé:

Les études sur le rôle des mycoplasmes génitaux (GM) dans les cancers gynécologiques (GC) tels que ceux du col de l'utérus, de l'endomètre, de l'ovaire, du vagin, de la vulve et des trompes de Fallope sont limitées. Cette revue a été menée pour évaluer une association entre GM et GC. L'étude systématique a été menée conformément aux directives PRISMA sur des bases de données en ligne, notamment Embase, Google Scholar, PubMed, Scopus et Web of Science, du début à août 2022. Nous avons inclus des études transversales et cas-témoins examinant un lien possible avec une infection GM et le développement de GC, et toutes les études fondées sur des preuves avec une association

probable entre l'infection GM et l'incidence de GC ont été étudiées. Les critères de sélection ont facilité l'identification, le dépistage et l'évaluation du risque de biais. Treize études présentant un risque de biais au moins modéré ont été incluses. Les GM les plus couramment associés étaient *Mycoplasma genitalium* suivi de *Ureaplasma urealyticum* et *Mycoplasma hominis*. Des études ont rapporté une progression de la maladie avec les GM, plus particulièrement dans les cas de co-infection. Les GC les plus associés étaient cervical, ovarien et endométrial. L'infection par *U. urealyticum, M. hominis* et *M. genitalium* était associée au cancer du col de l'utérus (OR 1,31-1,41), et *M. hominis* et *M. genitalium* présentaient un risque associé de cancer de l'ovaire (RR 0,93-1,92) et de l'endomètre (OR 1,36-2.07). Aucune association n'a été trouvée avec le cancer du vagin, de la vulve et des trompes de Fallope.

Mots clés: Mycoplasmes génitaux; cancer gynécologique; cancer du col de l'utérus; association; infection

Introduction:

Gynaecologic cancer (GC) is reported as the most prevalent amongst other cancers in women, with significant morbidity and mortality, accounting for 25% of cancer deaths in women globally (1). Gynaecologic cancers primarily are cancers of the reproductive organs of women; cervix, vulva, vagina, uterus, ovaries and fallopian tubes. Of these, cervical, ovarian and uterine cancers are the most prevalent. Annually, 881,000 new cervical, 265,000 new ovarian and 89,300 new uterine cancer cases with deaths, are reported (2,3). The principal origins of gynaecologic cancer are still unclear, however the American Cancer Society (ACS) showed the role infectious agents in gynaecologic cancer, increasing the risk of gynaecologic cancer by 20% (3).

Cancer is usually characterized by uncontrolled growth of abnormal cells in human tissues, which are usually excessive such that they metastasize, invade and spread to other organs or tissues of the body, causing life-threatening pathological situations in most cases. Studies have attributed causes of cancer to genetics, hereditary factors and carcinogens (4,5). Microorganisms such as viruses and genital mycoplasma have been studied and their link with cervical and other forms of gynecologic cancers have been established (6-10). The potential ability of atypical bacteria such as mycoplasmas in the invasion of host cells and further promotion of cellular transmission, as a precursor to cancer pathogenesis, have since been studied from inception in the 1950s (8,11-14). *In vitro* investigations of genital mycoplasmas for potential malignancy in host cells have been carried out using animal models and human cells, resulting in variations of cell transformation (15).

Mycoplasma are bacteria species with very small size and lacking cell wall. The term 'genital mycoplasmas' (GM) denotes a group of Mycoplasma species isolated from the genitals of asymptomatic sexually active individuals of both males and females. Consequently, the relatively high prevalence of these species among asymptomatic females have been reported (16). Mycoplasmas have also been strongly associated with infertility in women of reproductive age, as they were found to be more prevalent in these group of women than in fertile women of reproductive age (17,18). Mycoplasma co-infection and its contribution to onco-pathogenesis in cervical cancer have been recently studied in Africa (18). Apart from significant association with cervical cancer (OR 1.31, 95% CI 1.61-1.49; OR 1.41, 95% CI 1.10-1.99) reported in many studies (25-28), these organisms could play a role in other gynecological cancer such as endometrial cancer with significant association ($p< 0.0001$) reported in many studies (8,10, 20-24) as well as with ovarian cancer (RR 0.93, 95% CI 0.70-1.23; RR 1.92, 95% CI 0.78-4.72) also reported in some studies (7,10,29).

Some authors have reported GM involvement in some other clinically important gynaecologic pathologies such as bacterial vaginosis (30), cervicitis (31), pelvic inflammatory disease (32,33), salpingitis, uterine myomas and endometritis (7,34). Colonization of genitals by *Mycoplasma* and *Ureaplasma* have notably been linked with human papillomavirus (HPV) and pathogenesis (7,9). Although studies have not thoroughly investigated the prevalence of such co-infections, intra-epithelial lesions have been shown to play a mediating role in this co-infection (7,29). Chief amongst the bacterial agents involved in the pathogenesis of GM-associated genital malignancy are *M. genitalium* (35), *M. hominis,* and *U. urealyticum* (7,36-38).

Current diagnosis of GCs is available for only cervical cancer which usually involves investigation for cancerous and/or pre-cancerous cervical cells. It may be advocated that Mycoplasma screening be also incorporated into test algorithms for women with suspected gynecological cancers since studies have shown strong association with presence of Mycoplasma in gynecological cancers such as ovarian cancer (39).

A good number of studies have shown a remarkable link between cancers such as gynecological cancers and genital mycoplasmas, although some of them remain inconclusive, while a few others infer that other factors may be considered in mycoplasma-mediated genital oncology investigations. Understanding the role of genital mycoplasmas in chromosomal variability in gynaecologic malignancies/cancers development

may influence development of novel approaches to their prevention. Consequently, published articles in the scientific literature regarding the involvement and specific mechanism of genital Mycoplasma infection in the development of different types of gynaecologic cancer, were systematically evaluated in this study.

Methodology:

Search approach for the study

A systematic search approach was employed in accordance with the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines (40) across these scholarly databases; Embase, Google Scholar, PubMed, Scopus, and Web of Science from inception to August, 2022. The MeSH keywords used for the search included; *Mycoplasma* spp., *Ureaplasma* spp., *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Mycoplasma penetrans*, *Mycoplasma genitalium*, *Ureaplasma parvum*, genital mycoplasma, gynaecologic cancers, cervical, endometrial, ovarian, vulva, fallopian tube, vagina and gynaecologic tumours. The list of references for all retrieved articles similarly aided additional studies.

Systematic study selection:

The systematic selection of relevant articles included independent reviewers of two in number, to identify eligible studies using an established inclusion criteria for the study. In the instance of discrepancies, all authors considered the articles and a census reached. The inclusion criteria are; (i) journal articles published in English language and (ii) observational research articles including cross-sectional, case-control, cohort (retrospective and prospective) studies, containing study population, study type (hospital-based), type of gynaecological cancer, and genital mycoplasma infections (*Mycoplasma* spp., *Ureaplasma* spp., or both). Furthermore, review articles with systematic approach were also included.

Excluded from the study were journal articles not published in English language; congress/conference abstracts; article types such as letter to editor, case report with unstandardized methodology or inadequate data; studies with reported bacterial infections other than genital mycoplasma or viral infection in gynaecological cancer; and articles that considered an association between genital mycoplasma infection and other diseases except gynaecological cancer.

Quality assessment of included studies and data extraction:

The included studies were assessed for quality according to the Joanna Briggs Institute (JBI) critical appraisal (41). The study data assembled included characteristics such as first author, publication year, country, sample size (number of cases and/or control group), genital mycoplasma infection, frequency, diagnostic method, measures of association and participants' demographics.

Data synthesis and risk bias assessment:

Because the included studies had a low level of heterogeneity in study design, we employed a descriptive approach in analysing the data obtained. The assessment of risk of bias for included studies was done with the Cochrane Risk of Bias software with Risk of Bias of Exposures (ROBINS-E) applied for non-randomized studies (42). Each author assessed individual study within the risk of bias domain. Where discrepancy ensue, a consensus conclusion was attained. The bias risks for each domain were recorded accordingly.

Results:

Eligible studies:

Of 129 full-text articles assessed for eligibility, 116 were excluded based on the exclusion criteria. A total of 13 studies were included for data synthesis and risk bias assessment (Fig 1). Publication date for eligible studies was 1996 to 2020. Country of origin of the articles varied, with USA (n=4) having the highest number of included articles, followed by China (n=2), and Korea (n=2) (Table 1). In these studies, infections with GM were mostly assayed with polymerase chain reaction (PCR-ELISA, PCR 16SrRNA, qM-PCR and q-PCR-RT). However, culture, serology, lipid associated membrane protein enzyme immunoassay (LAMP-EIA) and Mycoplasma kit were also used. Furthermore, advanced high-throughput methodology such as metagenomic sequencing and PathoChip Array were also used.

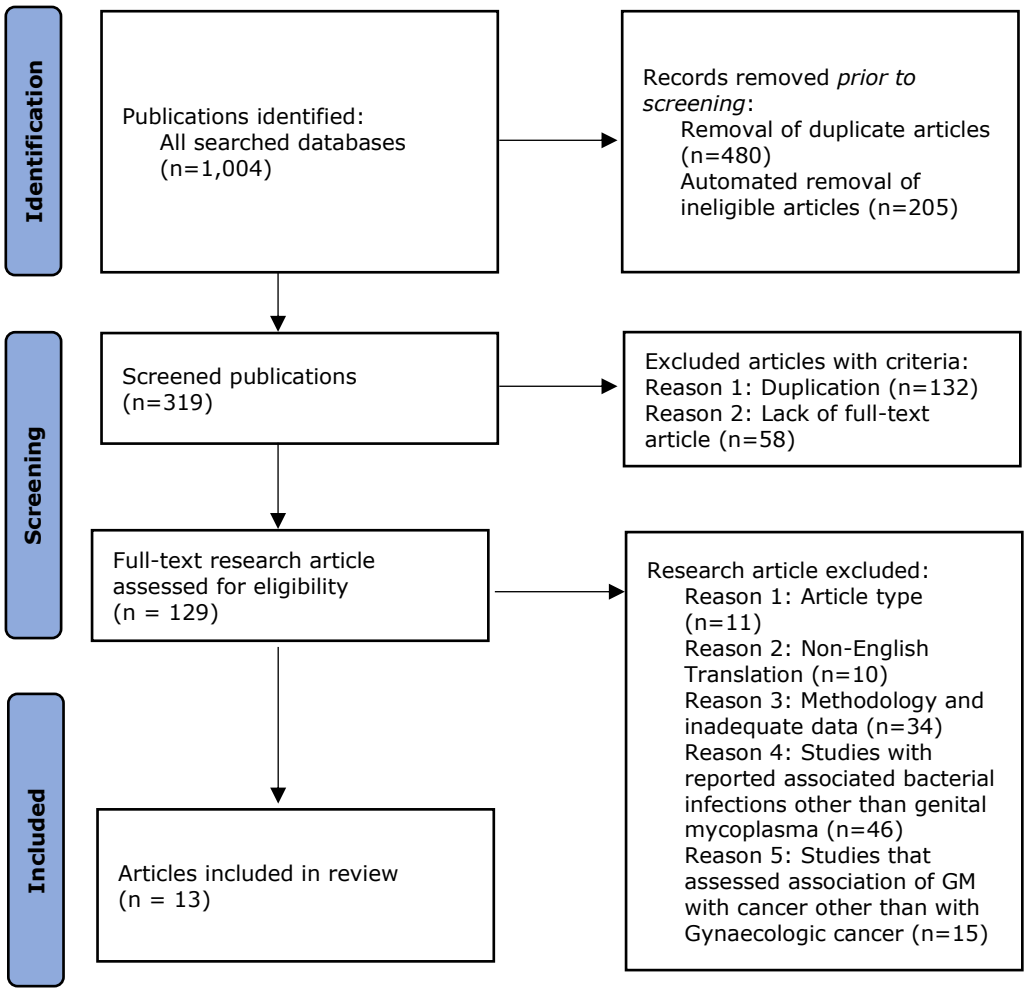

Fig 1: PRISMA flow chart with identification, screening and inclusion of articles

Associations with demographics and genital mycoplasma induced gynaecological cancer:

In the 13 included studies, data from 10,255 patients of different nationality with gynaecologic cancer (cervical, ovarian, endometrial vulva, vagina and fallopian tube) were reviewed. Patients diagnosed with GM-induced cancer were all sexually active with age ranging from 18 to 73 years. Sexually active young women (30–45 years) were also reported (19). Walther-António et al., (21) reported a significant association of GC with older women (58-71 years; $p < 0.0001$).

Other variables that could further predispose the patients to GM-induced gynaecologic cancer were also observed in the study. Walther-António et al., (21) reported significant association with post-menopausal (p=0.0034), but no association with history of diabetes (p= 0.621) and smoking status (p=0.5911). Only few authors studied these variables (Table 1).

Associations with cervical cancer:

The first cross-sectional study examined the cervical brush sample swabs of 1060 women (43). These samples were analysed using 16S metagenomic sequencing to determine the association of GM in each cervical cancer patient. Results obtained by the authors showed that cervical cancer patients with GM were significantly more prevalent in HPV patients. Abundance in *M. hominis* count significantly increased among patients with cervical cancer lesions. Overall, distribution of GM reported was *U. urealyticum* (51.4%), *M. hominis* (34%), and *M. genitalium* (2.3%) (43).

Secondly, the cervical microbiota of 4290 women (1452 HPV-positive group and 2838 HPV-negative group) were identified using molecular approach - PCR (9). The conventional

Table 1: Overview of articles included in study

Authored articles	Country	Year	Characteristics of the subjects						Sample type	Study type/ design	Sample size	Assay	GM/GC Type	Significant association
			Age range (years)	Nationality	Post-Meno-Pausal	Hypertension	Diabetes	Smoking						
Klein et al., (19)	Tanzania	2020	18-73	Tanzanian	Yes	NA	NA	NA	Cervical brush sample	HB/CS	1060	16S Metagenomics sequencing	MH, MG/CC	OR 2.1 $p < 0.0001$
Idahl et al., (44)	Germany	2020	30-81	European	Yes	NA	NA	NA	Blood samples	HB/CCS	2460	Serology	MG/OC	OR 1.36; 95% CI: 1.13–1.64
Fortner et al., (46)	USA	2019	34-81	American	Yes	NA	NA	NA	Plasma samples	HB/CCS	337	Serology	MG/OC	OR 2.07; 95% CI: 1.25–3.43; RR 1.92 (0.78-4.72)
Banerjee et al., (36)	USA	2017	NA	American	NA	NA	NA	NA	Ovarian cancer tissue	HB/CCS	99	PathoChip Array	GM/OC	$p < 0.05$
Liu et al., (9)	China	2016	18-66	Chinese	Yes	No	No	NA	Cervical brush specimen	HB/CCS	4290	PCR	UU/CC	OR 1.31 95% CI: 1.16-1.49
Walther-Antonio et al., (21)	USA	2016	≥ 18	Caucasian	Yes	Yes	No	No	Vaginal, cervical, fallopian, ovarian	HB/CS	31	16SrDNA V3-V5 region Mi-Sequecing	MH/EC	$p < 0.001$
Xiaolei et al., (26)	China	2014	20-67	Chinese	Yes	NA	NA	NA	Cervical secretion	HB/CCS	233	qPCR (Fluorescence)	UU/CC	$p = 0.002$
Debon and McGowin (25)	Korea	2014	20-70	Louisiana	Yes	NA	NA	NA	Cervical Secretion	HB/CCS	347	qPCR-RT	MG/CC	$p < 0.05$
Choi et al., (27)	Korea	2014	20-45	Korean	No	NA	NA	NA	Cervico-vaginal secretion	HB/CS	714	PCR	MH,MG, UU/CC	$p = 0.054$
Farag et al., (43)	Egypt	2013	20-48	Egyptian	No	NA	NA	Yes	Endo-cervix and posterior vaginal sample	HB/CS	300	Mycoplasma kit/Pap smear cytology	MH, UU/CC	OR 1.41-1.51; 95% CI: 1.10-1.99
Idahl et al., (45)	Sweden	2011	18-87	Swedish	Yes	NA	NA	Yes	Plasma sample	HB/CCS	118	LAMP-EIA	MG/OC	RR 0.93 95% CI: 0.70-1.23; p=0.01
Lukic et al., (28)	Italy	2006	18-50	Italian	No	NA	NA	NA	Exo-endo-cervical and vaginal sample	HB/CCS	239	Culture	UU/CC	NA
Chan et al., (37)	USA	1996	NA	NA	NA	NA	NA	NA	Achived human ovarian tisuue	HB/CCS	27	Combined PCR-ELISA	GM/OC	NA

NA = not avaliable; HB = hospital-based; CS = Cross sectional study; CCS = Case-control study; MG = *Mycoplasma genitalium*; UU = *Ureaplasma urealyticum*; MH = *Mycoplasma hominis*; GM = Genital Mycoplasma; GC = Gynecologic cancer; EC = Endometrial Cancer; OC = Ovarian Cancer; CC = Cervical Cancer; qPCR = Quantitative Polymerase chain reaction; PCR = Polymerase chain reaction; LAMP-EIA = Lipid associated membrane protein enzyme immunoassay; PCR-ELISA = Polymearse chain reaction – enzyme-linked immunosorbent assay

PCR result showed significant association of *U. urealyticum* in the microbiota of HPV-positive patients (AOR 1.18; 95% CI 1.04–1.34) and *U. urealyticum* were found in significantly higher frequencies (58.2%) among the HPV-positive women (9). High prevalence of *U. urealyticum* (49.3-83%) was found to be associated with the grade of cytological cervical lesions (squamous abnormalities) in 533 participants (19,26-28). *Ureaplasma urealyticum* was significantly associated (p<0.05) with risk of cervical cancer in high-grade squamous lesion (HSIL) (57.5%-65%), atypical squamous cervical cells of undetermined significance (ASCUS) (27%-30.43%) and low-grade squamous intra-epithelial lesion (LSIL) (14%-36.59%). The high presence of *U. urealyticum* in these studies is in association with HPV, which is consequently, a potential co-factor for HPV-induced precancerous and cervical cancer. *M. hominis* was found to be more prevalent than *M. genitalium* among women with cervical cancer (26,27), however, *M. genitalium* was significantly prevalent in women with severe cervical inflammation.

Association with ovarian cancer:

Mycoplasma genitalium antibodies was identified in 11.76% of 68 females with epithelial ovarian cancer using multiplex fluorescent bead-based serology (lipid-associated membrane protein-enzyme immunoassay) (44). The result showed a significant association (RR 0.93 (95% CI 0.70-1.23). A similar study found significant association (p=0.01) following detection of *M. genitalium* IgG antibodies among women with borderline ovarian tumours (45), although the association in this study was due to a type 1 error from Bonferroni correlation which reduced the significance of the finding. A significant association was reported from the analysis of plasma samples of 336 ovarian cancer patients with showed presence of MgPa-N-Terminus and MgPa antibodies of *M. genitalium* in 17% of the study population (RR 1.92, 95% CI 0.78-4.72) (46), although *C. trachomatis* seropositivity was associated with higher risk of ovarian cancer (RR 2.07, 95% CI 1.25-3.43), similar for invasive serous, and borderline tumours.

Using a molecular approach such as combined PCR-ELISA method, genital mycoplasma was detected in 59.3% of the study population using a genus-specific primer (37). Further analysis with a specie-specific primer detected *U. urealyticum* as the dominant specie. An analysis of 99 ovarian cancer tissue samples with Pan-Pathogen Array (PathoChip) technology combined with high throughput sequencing, detected an abundance (74%) of genital *Mycoplasma* spp among the microbiome of ovarian cancer patients (p <0.05) (36). However, *Mycoplasma* and *Ureaplasma* (Tenericutes) are not higher on the list of bacterial signatures in ovarian cancer when compared to Proteobacteria and Firmicutes.

Association with endometrial cancer:

The abundance of *U. urealyticum* and *M. hominis* was reported among women with benign uterine disease, uterine hyperplasia or any stage of endometrial cancer undergoing hysterectomy in a case-control study (21). Sequencing result revealed that these women had progressive PID which led to chronic inflammation and subsequently change in uterine endothelial cells (carcinogenesis).

Discussion:

This systematic review of studies intended to assess current trend of the association between GM and GC, with their aetiological and causative factors in different geographical locations. The finding associating the risk of endometrial cancer with variables such as hypertension in American women by Walther-Antonio et al., (21) may not be entirely in concurrence with other findings due to the difference in methodology adopted by the different researchers who used serology (46) and pathochip (36) as their prefered methods of analysis during their research with different study particpants from different geographical regions.

The role of *U. urealyticum* as an important factor in the promotion of gynaecological pathological processes such as cervical cancer can no longer be overlooked even at the global level, this is because studies by Choi and Roh (27) conducted in Korea, together with studies conducted with Chinese participants by Xiaolei et al., (26) were both in concurrence with a previous study conducted in Europe by Lukic et al., (28) as they all detected *U. urealytium* associated with cervical cancer. On this basis, global documentation of this organism as a gynaecological cancer-associated atypical microbe has become imperative, as it has shown to promote morbidity in women with gynaeological cytopathologies regardless of race, ethnicity and the geographical location of the patient.

Although most of the articles reviewed were mostly studies done from outside Africa, a recent study done in Africa by Klein et al., (19) with Tanzanian women as study participants using molecular methods revealed the link between *Mycoplasma* and cervical cancer in the study participants. The scarcity of African-based studies may not be unconnected with the fact that the use of molecular techniques for diagno-

stic and research purpose is a relatively new development in Black Africa and could be costly for both routine and research investigations, with scarcity of trained personnels an important factor, compared to other continent and climes (16). Another factor that may be contributory to the dearth of case-studies in sub-saharan Africa in comparison to other climes, could be the reluctance of patients in seeking hospital-based care. Many patients in Africa prefer to seek traditional remedies such as polyherbals as their primary source of care, some of these polyherbal have been proven to be contaminated above safe levels (47). Prior to this study, a previous study conducted in Egypt with women of middle-eastern origin as study subjects did not deploy molecular-based methodology, it showed a connection between cervical cancer and bacteria such as *M. hominis* and *M. genitalium*.

Scientific reviews have proven that the link between mycoplasma and cancer can no longer be considered a mere coincidence or happenstance by the global medico-scientific community, this link has been noted since 1950 when *Mycoplasma* were first noted in patients with leukemia (11). The predispostion of older and post-menopausal women as seen in this review, may also be attributed to the lowering of immunity as the women progressively age into geriatic status. It is also understood that immunocompentence dwindles as women become older (48) and may also contribute to dybiosis in the cervico-vaginal milieu of women. Dysbiosis have also been shown to be linked to many diseases such as cancer (49). Immunological factors such as pro-inflammatory cytokines which promotes inflammation in the cervicovaginal epithelium many also be upregulated during dysbiosis and displacement of friendly commensals and probiotic bacteria such as *Lactobacillus acidophilus* and *Lactobacillus crispatus* (50), who help to physiologically protect the genito-urinary tract of women from pathogenic and harmful bacteria colonization, by encouraging the growth and survival of bacteria such as mycoplasma which have been linked to some gynaecological cancers (51).

A positive concordance between molecular-based test and standard culture technique in the detected genito-urinary tract infection was noted at 98%. Notwithstanding this postulation, molecular-based test possess several features that makes it relatively beneficial to timely diagnosis and management of fastidious genito-urinary pathogens such as *Mycoplasma* and *Ureaplasm*a. This test has shown to be effective in the utilization as it can identify fastidious bacteria, provide faster assessment of antibiotic susceptibility, track drug resistance patterns and help identify factors that may affect therapy and re-infection of gynaecological microbial diseases (52,53). It is therefore hoped that since most of the articles reviewed in this study were based on molecular studies, this therefore should confer a substantial level of uniformity on the methodology of research works conducted, ensuring reproduceablity of the studies in this systematic review.

Conclusion:

Based on this review, it is understood that mycoplasma-associated gynaecoloical cancers are not specific to any geographical region and they have been detected in women of almost all race and regions, with both *Mycoplasma* and *Ureaplasma* being associated with increased risk of cervial cancer, while only *Mycoplasma* have shown association with increased risks of ovarian and endometrial cancers in women. Molecular-based test for routine investigation of genital mycoplasma in gynaecological pathologies should be encouraged in hospitals.

Contribution of authors:

CCE conceptualized the study; NRA and CVU collected and analyzed the data; IOU, CBO and ISE were involved in analysis of data; and CCE and CVN produced the manuscript draft. All authors approved the final manuscript.

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Conflict of interest:

Authors declare no conflict of interest

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